Diamond Electrodes For Neurodynamic Studies in *Aplysia californica*

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As part of an ongoing effort to demonstrate that diamond is a versatile electrode material for biological applications, we present our progress in development of diamond electrodes for study of neurodynamics in an animal model, *Aplysia californica*. Diamond provides a unique opportunity to integrate neural stimulation and sensing in the same implantable device. Data from several parallel studies are presented: *in vitro* measurement of dopamine and serotonin concentration, measurement of electrical activity, and neural stimulation. Using diamond microelectrodes, changes of the *Aplysia californica's* feeding patterns are being studied as a function of concentration of dopamine (acting as a neurotransmitter) or serotonin (acting as a neuromodulator).

1 Background and Motivation

We are exploring the advantages of diamond electrodes using a specific, relatively well-defined neural circuit that provides known controls for comparison and minimizes surgical challenges. At the same time, data from this newly developed diamond device may provide new insights into behavioral functions of this neural circuit. *Aplysia californica*, a marine mollusk, is a commonly used animal model for neurodynamic studies. Our focus is on the buccal mass, *i.e.*, the muscular structure for feeding, and the buccal ganglion, a collection of cells that control the feeding motor patterns of *Aplysia californica*. The somata (cell bodies) of the buccal motor neurons are large (~50-150 microns), identifiable, and readily accessible; thus, it is possible to obtain electrophysiology recordings and monitor chemical release from a single cell. In addition, neural activity can often be directly correlated with muscular response; *e.g.*, patterns of buccal nerve recordings *in vivo* (corresponding to recordings of multiple neurons firing) can distinguish between the three fundamental feeding behaviors: biting, rejecting, and swallowing (Ref. 1). *Aplysia* provides unique advantages for development of an integrated device since all three aspects – stimulation, electrical activity and neurotransmitter sensing – can be probed at the neuron cell body.

Of specific interest for these studies are the B65 neuron and the metacerebral cell (MCC). The B65 neuron is located in the collection of nerve cells controlling the feeding apparatus, the buccal ganglion; it has been shown to enhance rejection motor patterns, and is known to contain dopamine, which acts as a modulatory neurotransmitter (Ref. 2). The MCC is located in the cerebral ganglion (a more encephalized collection of nerve cells that controls higher order behavioral choice); it has multiple roles in feeding behavior that, in part, involve the release of serotonin, a neuromodulator, throughout the buccal mass. The MCC contains approximately 100 μM serotonin in the soma and 4% is released with the generation of an action potential (Ref. 3). Through use of real-time detection with diamond electrodes, we seek to determine the release site and concentration of (a) *dopamine* from the B65 neuron and (b) *serotonin* from the MCC during a normal feeding pattern.

2 Methods

Microelectrodes (~30 μm diameter) were fabricated using hot-filament-assisted CVD techniques to deposit boron-doped diamond onto a tungsten microelectrode; for details, see Ref. 4. Flow cell calibrations were used to estimate the detection limits; fast scan cyclic voltammetry (FSCV) was the detection method. **Figure 1** shows an example flow cell calibration for a diamond microelectrode; 1 nM dopamine (**Fig. 1a**) and 50 nM serotonin (**Fig. 1b**) were detected (Ref 4).

The buccal ganglion is encased in an insulating layer (sheath) that must be surgically removed (desheathed) to allow study of the chemical transmission between cells. Intracellular recordings (*i.e.*, within the cell) were conducted by inserting a sharp glass pipette (<1 micron) into the soma and recording membrane potential versus time. Extracellular measurements, *i.e.*, with the sheath still intact, are less invasive and thus more desirable for *in vivo* studies. Using extracellular suction electrodes, nerve activity was measured (*i.e.*, the combined signal representing activity in axons from various neurons). Individual neurons are distinguished by their signal amplitude on the nerve. For extracellular recordings from a single neuron with the sheath intact, a single electrode was lowered over the cell of interest and the time-varying voltage recorded (**Fig. 2a**). In all preparations, *Aplysia* saline was used. Although our ultimate interest is in the B65 and MCC neurons, the neurons B4/B5 were used for preliminary studies because they are the most surgically accessible, and our group has extensive experience in studying their function.

3 Results

We are currently using diamond electrodes for *in vitro* recordings and stimulation. Preliminary, extracellular recording and stimulation were conducted from a single neuron, B4 or B5 (two electrically coupled neighboring neurons), through the sheath (**Fig. 2**). For extracellular recording from B4/B5, a one-to-one relationship was observed on the diamond electrode, so that it was clearly recording from one neuron, whereas the suction electrode on the nerve recorded two signals, reflecting activity in both neurons (**Fig. 2b**). For extracellular stimulation, a one-to-one relationship was also observed, indicating that one neuron was stimulated without activating neighboring cells (**Fig. 2c**). Also, rejection (neural) patterns from a desheathed ganglion were obtained, and will be used to identify the B65 neuron for our dopamine studies.

We are also exploring measurement of serotonin release from the MCC. The MCC releases serotonin at the soma and throughout the buccal mass, including the I2 muscle, the primary muscle for protraction (Ref 5). Sutton *et al.* (Ref. 6) have established that exogenously added serotonin enhances I2's contractile strength, thus indicating that serotonin plays a direct role in the behavioral function of I2.

In the future, we will integrate FSCV and neural recording at the same electrode; if successful, an array will be designed to record from various sites throughout the buccal ganglion, to help map the chemical and electrical signals *in vivo* during a complete feeding behavior.

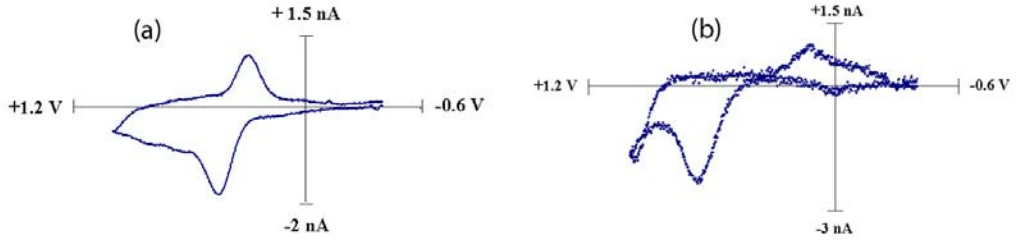

Figure 1: Cyclic voltammograms, at 300 V/s in HEPES buffer of (a) 1 nM dopamine and (b) 50 nM serotonin.

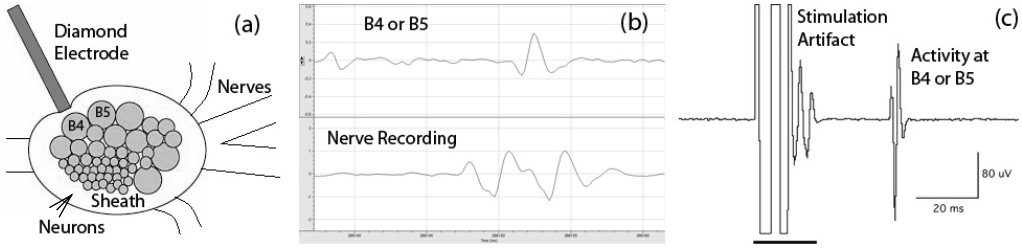

Figure 2: (a) Schematic of the extracellular stimulation/recording method, with the diamond electrode pressed into the sheath over the cells of interest for proper recording/stimulation. (b) Extracellular recording above B4 or B5. The diamond electrode (*top*) recorded single-cell activity, while a suction electrode on the nerve (*bottom)* recorded activity from both cells. (c) Extracellular stimulation at B4 or B5 produced a clear signal after stimulation.

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